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Research Paper

Interaction of Ibuprofen and Other Structurally Related NSAIDs with the Sodium-Coupled Monocarboxylate Transporter SMCT1 (SLC5A8)

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Purpose. Sodium-coupled monocarboxylate transporter 1 (SMCT1) is a Na^+-coupled transporter for monocarboxylates. Many nonsteroidal anti-inflammatory drugs (NSAIDs) are monocarboxylates. Therefore, we investigated the interaction of these drugs with human SMCT1 (hSMCT1).
Methods. We expressed hSMCT1 in a mammalian cell line and in *Xenopus laevis* oocytes and used the uptake of nicotinate and propionate-induced currents to monitor its transport function, respectively. We also used [^{14}C]-nicotinate and [^{3}H]-ibuprofen for direct measurements of uptake in oocytes.
Results. In mammalian cells, hSMCT1-mediated nicotinate uptake was inhibited by ibuprofen and other structurally related NSAIDs. The inhibition was Na^+ dependent. With ibuprofen, the concentration necessary for 50% inhibition was 64 ± 16 μM. In oocytes, the transport function of hSMCT1 was associated with inward currents in the presence of propionate. Under identical conditions, ibuprofen and other structurally related NSAIDs failed to induce inward currents. However, these compounds blocked propionate-induced currents. With ibuprofen, the blockade was dose dependent, Na^+ dependent, and competitive. However, there was no uptake of [^{3}H]-ibuprofen into oocytes expressing hSMCT1, although the uptake of [^{14}C]-nicotinate was demonstrable under identical conditions.
Conclusions. Ibuprofen and other structurally related NSAIDs interact with hSMCT1 as blockers of its transport function rather than as its transportable substrates.

KEY WORDS: blocker; electrophysiology; ibuprofen; sodium dependence; SMCT1.

INTRODUCTION

Sodium-coupled monocarboxylate transporter 1 (*SMCT1*; *SLC5A8*) was recently identified as a candidate tumor suppressor gene in humans that is silenced by methylation in colon cancer (1). Short-chain fatty acids (e.g., acetate, propionate, butyrate), lactate, and nicotinate are recognized by the transporter as transportable substrates (2–5). The SMCT1-mediated transport is Na^+ dependent and electrogenic. SMCT1 is expressed in the colon, kidney, and thyroid gland, and the biologic role of the transporter may vary from tissue to tissue (6). In the colon, the transporter functions to absorb short-chain fatty acids from the lumen; these fatty acids are generated at high concentrations in the colonic lumen by bacterial fermentation of dietary fiber (7,8). In the kidney, the physiologic role of the transporter is to reabsorb lactate and nicotinate (4,5). In the thyroid gland, the transporter may participate in the release of iodide from the follicular cells into the colloidal lumen (9) (Gopal *et al.*, unpublished data). Because butyrate is an inhibitor of histone deacetylases (10), the ability of SMCT1 to mediate the concentrative accumulation of this fatty acid in colonocytes may underlie the tumor suppressive function of this transporter in the colon (11).

Plasma membrane transporters play a significant role in determining the bioavailability, therapeutic efficacy, and pharmacokinetics of a variety of drugs (12–15). The interaction of plasma membrane transporters with drugs has pharmacologic, clinical, and therapeutic relevance. Such interactions may influence not only the therapeutic efficacy, biologic half-life, and tissue distribution of drugs but also may underlie some of the undesirable side effects associated with the use of these drugs. Therefore, given the multitude of biologically important functions of SMCT1, it is critical to determine if any of the widely used therapeutic agents interacts with this transporter. A recent study by Coady *et al.* (3) indicated that human SMCT1 (hSMCT1) is inhibitable by ibuprofen, a widely used nonsteroidal anti-inflammatory drug (NSAID). Ibuprofen possesses a carboxylate group, and

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ABBREVIATIONS: HRPE, human retinal pigment epithelial; MCT, monocarboxylate transporter; NMDG, *N*-methyl-D-glucamine; NSAID, nonsteroidal anti-inflammatory drug; SMCT, sodium-coupled monocarboxylate transporter.

this structural component might be the basis for its ability to interact with the transporter. This was a very interesting finding, but several questions remain unanswered. Is ibuprofen a transportable substrate for SMCT1? Or, does this drug function as a blocker of the transporter? What is the role of Na^+ in the interaction between the transporter and ibuprofen? Does the drug interact with the transporter via the substrate-binding site? Do other structurally related NSAIDs also interact with the transporter? The present investigation was carried out to answer these important questions.

MATERIALS AND METHODS

Materials

[^{14}C]-Nicotinate (specific radioactivity, 55 mCi/mmol) and [^{3}H]-ibuprofen (specific radioactivity, 0.5 Ci/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Propionate, ibuprofen, ketoprofen, fenoprofen, and naproxen were purchased from Sigma (St. Louis, MO, USA). The molecular cloning of functional hSMCT1 has been described previously (2).

Functional Expression of hSMCT1 cDNA in Human Retinal Pigment Epithelial Cells

hSMCT1 cDNA was expressed functionally in human retinal pigment epithelial (HRPE) cells using the vaccinia virus expression technique (4,5). Uptake of [^{14}C]-nicotinate (15 or 30 μM) was measured with a 5-min incubation (linear uptake rates) at 37°C, 12–15 h posttransfection. HRPE cells transfected with the empty vector were used to measure the background transport activity, which might occur via diffusion or carrier-mediated pathways such as the monocarboxylate transporters (MCTs) (16). These cells do not possess any detectable endogenous SMCT1-like transport activity (4). Uptake measurements in vector- and cDNA-transfected cells were always made in parallel under identical experimental conditions. The hSMCT1-mediated transport was determined by subtracting the transport values measured in vector-transfected cells from the transport values measured in cDNA-transfected cells. The uptake buffer was 25 mM HEPES/Tris (pH 7.5), containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. When transport measurements were made in the absence of Na^+, NaCl in the transport buffer was replaced with an equimolar concentration of *N*-methyl-d-glucamine (NMDG) chloride. The IC_{50} values (i.e., concentrations of inhibitors necessary for 50% inhibition) were calculated from dose-dependence studies. The Na^+ dependence of inhibition by ibuprofen on hSMCT1-mediated nicotinate transport was determined by monitoring the transport in the absence or presence of the inhibitor, but at different concentrations of Na^+.

Functional Expression of hSMCT1 in *Xenopus laevis* Oocytes

Capped cRNA from hSMCT1 cDNA (cloned in pGH19, an *X. laevis* oocyte expression vector) was synthesized using the mMESSAGE-mMACHINE kit (Ambion, Austin, TX, USA). Mature oocytes from *X. laevis* were isolated by treatment with collagenase A (1.6 mg/mL), manually defolliculated and maintained at 18°C in modified Barth's medium, supplemented with 25 μg/mL gentamicin as described previously (2). The research adhered to the "Principles of

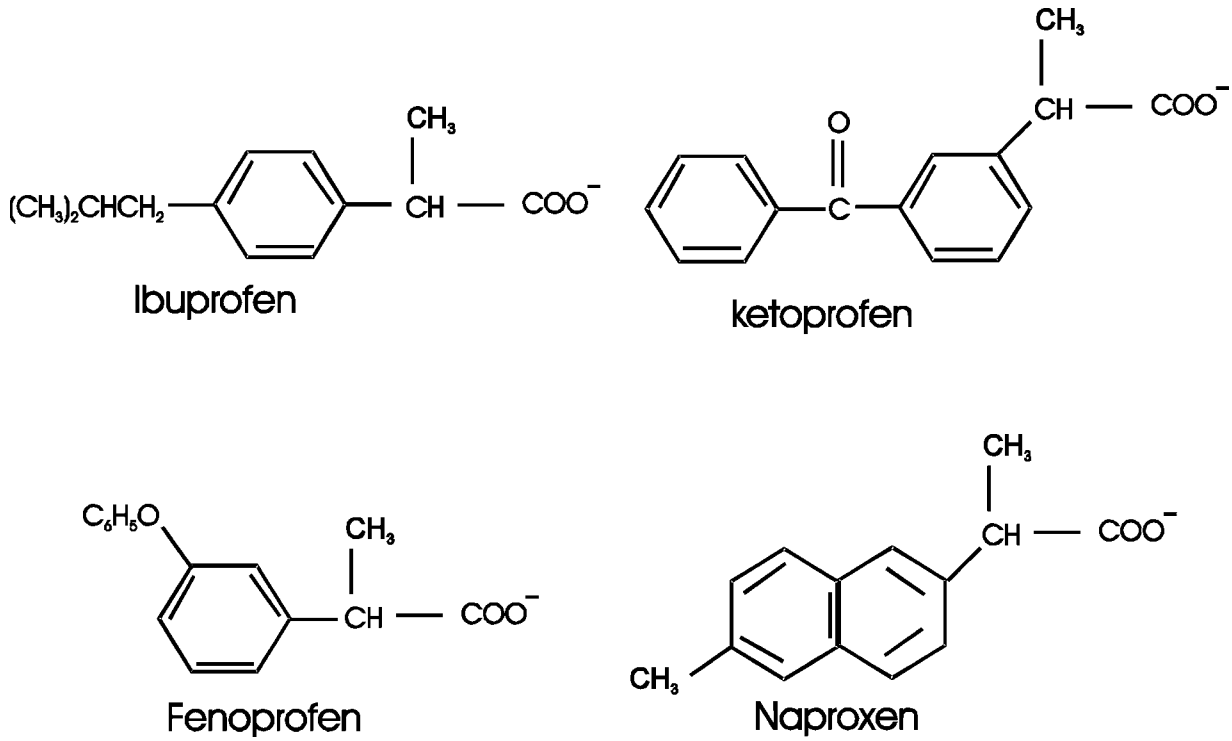

Fig. 1. Chemical structures of ibuprofen, ketoprofen, fenoprofen and naproxen.

Table I. Inhibition of hSMCT1-Mediated Nicotinate Uptake in HRPE Cells by NSAIDs

Inhibitors	[^{14}C]-Nicotinate uptake [pmol (10^6 cells)$^{-1}$ min^{-1}]	% Control
Control	91.9 ± 2.4	100
Ibuprofen	20.9 ± 2.2	23
Ketoprofen	47.0 ± 0.7	51
Fenoprofen	28.1 ± 1.1	31
Naproxen	62.9 ± 2.3	69

hSMCT1 cDNA was expressed functionally in HRPE cells by the vaccinia virus expression technique. Transport activity of hSMCT1 was monitored by the uptake of 30 μM [^{14}C]-nicotinate in the presence of NaCl (5-min incubation). Uptake was measured in the absence (control, 100%) and presence of 200 μM NSAIDs. Data (cDNA-specific uptake) are presented as means ± S.E.M.
NSAIDs = nonsteroidal anti-inflammatory drugs.

Laboratory Animal Care" (NIH publication #85-23, revised in 1985) and was approved by the institutional Committee for Animal Use in Research and Education. On the following day, oocytes were injected with 50 ng of cRNA. Water-injected oocytes served as control. The oocytes were used for electrophysiological studies 3–7 days after cRNA injection. Electrophysiological studies were performed by the two-microelectrode voltage-clamp method. Oocytes were perfused with a NaCl-containing buffer (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, pH 7.5), followed by the same buffer containing propionate and various NSAIDs. The membrane potential was clamped at −50 mV. The kinetics of ibuprofen effect on propionate-induced currents was analyzed by determining the inhibition in the presence of increasing concentrations of ibuprofen in the perfusion buffer. The Na^+ dependence of the ibuprofen effect on propionate-induced currents was analyzed by monitoring the ibuprofen-induced inhibition of the currents in the presence of increasing concentrations of Na^+. In these experiments, the osmolality of the perfusion buffer was maintained by the addition of appropriate concentrations of NMDG chloride. The influence of Cl^- on the ibuprofen effect was analyzed by monitoring the ibuprofen-dependent inhibition of propionate-induced currents in the presence of Na^+ but in the absence of Cl^-. The perfusion buffer for these studies was prepared by using gluconate salts of Na^+ and K^+ instead of chloride salts. To study the influence of pH, the ibuprofen-dependent inhibition of propionate-induced currents was monitored at pH 7.5 and at pH 6. The pH of the perfusion buffer was altered by adjusting the relative concentrations of MES, HEPES, and Tris. The $K_{0.5}$ value (i.e., concentration of ibuprofen necessary for 50% inhibition) was calculated from dose-dependence studies. Saturation kinetics of propionate-induced currents in the absence or presence of ibuprofen was analyzed by fitting the data to Michaelis-Menten equation, and the kinetic parameters, $K_{0.5}$ (concentration of propionate needed to induce half-maximal currents) and I_{max} (maximal current inducible by saturating concentrations of propionate), were determined. In the analysis of Na^+-dependence kinetics, the data were analyzed by the Hill equation to determine the Hill coefficient (h; the number of Na^+ ions involved in the interaction of ibuprofen with the transporter) and $K_{0.5}$ for Na^+ (the concentration of Na^+ necessary for half-maximal inhibition by ibuprofen). Uptake of radiolabeled nicotinate and ibuprofen into water-injected and hSMCT1-expressing oocytes was measured as described previously (4,5), using eight to ten oocytes for each measurement.

Data Analysis

Experiments with HRPE cells were repeated with three independent transfections, and transport measurements were made in duplicate each time. Electrophysiologic experiments were repeated with at least four different oocytes. Data are presented as means ± S.E.M. The kinetic parameters were determined using the computer program Sigma Plot, version 6.0 (SPSS, Chicago, IL, USA).

RESULTS

Studies using the Mammalian Cell Expression System

First, we investigated the interaction of hSMCT1 with ibuprofen and structurally related NSAIDs using the mammalian cell expression system in which the cloned hSMCT1 was expressed heterologously in HRPE cells. The function of hSMCT1 in these cells was monitored by the Na^+-dependent uptake of nicotinate. Ibuprofen and three other structurally related NSAIDs (ketoprofen, fenoprofen, and naproxen) (Fig. 1) were evaluated for their ability to compete with nicotinate for the uptake process mediated by hSMCT1. With nicotinate at a concentration of 30 μM, all four NSAIDs tested (concentration, 200 μM) showed significant inhibition of nicotinate uptake (Table I). The inhibition varied in the range of 30–80%, with ibuprofen and fenoprofen showing

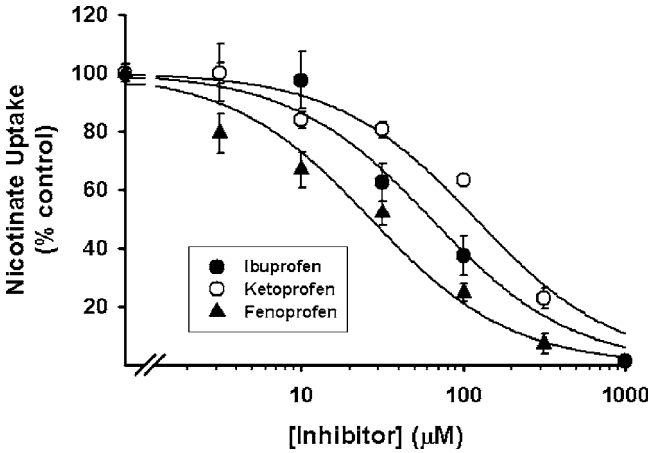

Fig. 2. Dose–response relationship for the inhibition of human sodium-coupled monocarboxylate transporter 1 (hSMCT1)-mediated nicotinate uptake by ibuprofen, ketoprofen, and fenoprofen in human retinal pigment epithelial (HRPE) cells. HRPE cells were transfected with either vector alone or hSMCT1 cDNA. Uptake of [^{14}C]-nicotinate (15 μM) was measured for 5 min in the presence of NaCl and in the presence of increasing concentrations of various nonsteroidal anti-inflammatory drugs (NSAIDs). Results shown (cDNA-specific uptake) are percentage of control uptake (100%) measured in the absence of inhibitors.

Table II. Na^+ Dependence of the Inhibitory Effect of Ibuprofen on hSMCT1-Mediated Nicotinate Uptake in HRPE Cells

	[^{14}C]-Nicotinate Uptake [pmol (10^6 cells)$^{-1}$ min^{-1}]			
[Na^+]	hSMCT1	pSPORT1	cDNA specific	% Control
10 mM (−)	7.0 ± 0.4	6.4 ± 0.5	0.6	100
10 mM (+)	6.5 ± 0.4	6.1 ± 0.5	0.4	67
50 mM (−)	16.3 ± 1.6	6.7 ± 0.7	9.6	100
50 mM (+)	10.4 ± 0.8	5.8 ± 0.3	4.6	48
140 mM (−)	40.6 ± 3.6	7.7 ± 0.7	32.9	100
140 mM (+)	14.8 ± 1.2	6.9 ± 0.6	7.9	24

Uptake of [^{14}C]-nicotinate (15 μM) was measured in HRPE cells transfected with either vector alone (pSPORT1) or vector hSMCT1 cDNA construct (hSMCT1). Uptake measurements were made in the presence of three different concentrations (10, 50, and 140 mM) of Na^+ with (+) or without (−) 200 μM ibuprofen (5-min incubation). Osmolality of the uptake buffer was maintained by the addition of appropriate concentrations of *N*-methyl-D-glucamine chloride as a substitute for NaCl. Data are presented as means ± S.E.M.

considerably greater inhibitory potencies compared with ketoprofen and naproxen. We then analyzed the dose–response relationship for the inhibition of hSMCT1-mediated uptake of nicotinate by ibuprofen, ketoprofen, and fenoprofen (Fig. 2). The IC_{50} values (i.e., concentration necessary for 50% inhibition) calculated from these studies for these three compounds were 64 ± 16, 119 ± 30, and 27 ± 6 μM, respectively. We then investigated whether there is any role for Na^+ in the inhibition of hSMCT1 activity by ibuprofen. We compared the magnitude of ibuprofen-induced inhibition of hSMCT1-mediated nicotinate uptake at three different concentrations of Na^+ (Table II). With nicotinate at a concentration of 15 μM and ibuprofen at a concentration of 200 μM, the inhibition was 33% at 10 mM Na^+, which increased to 52% at 50 mM Na^+ and to 76% at 140 mM Na^+. These data show that the inhibitory potency of ibuprofen is enhanced with increasing concentrations of Na^+.

Studies Using the *X. laevis* Oocyte Expression System

The results obtained with the mammalian cell expression system show that ibuprofen and other structurally related NSAIDs interact with hSMCT1 as assessed by their abilities to inhibit nicotinate uptake via the transporter. However, these studies do not show whether or not these NSAIDs actually compete with nicotinate for the substrate-binding site and serve as transportable substrates for the transporter. It is possible that these NSAIDs block the uptake by competing with nicotinate for binding to the substrate-binding site of the transporter without themselves being transported across the membrane. To determine whether these compounds are transportable substrates for hSMCT1, direct measurements of their transport must be monitored using radiolabeled NSAIDs rather than merely demonstrating the ability of unlabeled NSAIDs to compete with nicotinate. Therefore, we used an alternative method to address this issue. The *X. laevis* oocyte expression system is capable of distinguishing whether a given compound is a transportable substrate or merely a blocker without the need for radiolabeled compounds. We expressed hSMCT1 in *X. laevis* oocytes and monitored its transport function by electrophysiological means using the two-microelectrode voltage-clamp method. If a given compound serves as a transportable substrate for hSMCT1, we should be able to detect Na^+-dependent inward currents induced by the compound in oocytes expressing the transporter. If a given compound is a blocker and not a transportable substrate, there will be no inward currents associated with the interaction between the compound and the transporter. With this rationale, we examined ibuprofen and the other three related NSAIDs for their ability to induce inward currents in the presence of Na^+ in oocytes expressing hSMCT1 (Fig. 3). First, we confirmed that hSMCT1 was expressed functionally in these oocytes by monitoring currents in the presence of propionate, a known transportable substrate for the transporter. This short-chain fatty acid induced marked inward currents in hSMCT1-expressing oocytes (180 ± 29 nA at a concentration of 2.5 mM) (Table III). Under identical conditions, neither ibuprofen nor any of the other related NSAIDs was able to

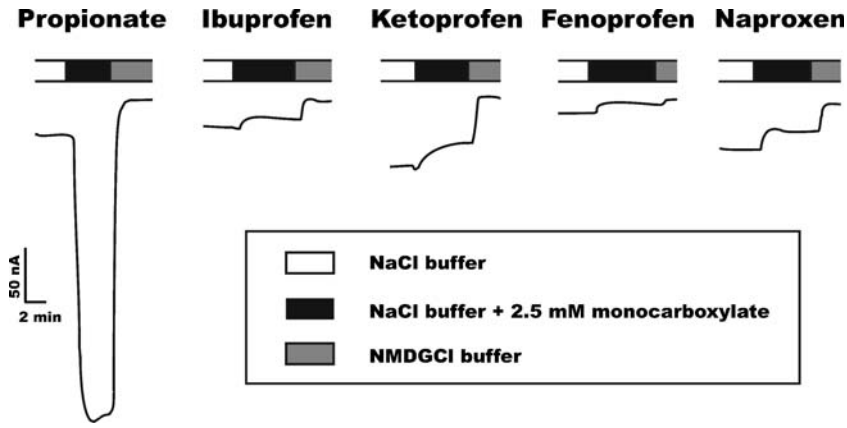

Fig. 3. Inward currents induced by propionate and NSAIDs in oocytes expressing hSMCT1. hSMCT1 was expressed in *Xenopus laevis* oocytes by cRNA injection. Substrate-induced currents were recorded in the presence of NaCl using the two-microelectrode voltage-clamp technique.

Table III. Currents Induced by Propionate and Different NSAIDs in *X. laevis* Oocytes Expressing hSMCT1

Substrate	Induced current (nA)
Propionate	−180 ± 29
Ibuprofen	6 ± 1
Ketoprofen	9 ± 1
Fenoprofen	−3 ± 8
Naproxen	5 ± 5

hSMCT1 was expressed in oocytes, and currents induced by different compounds (2.5 mM) were monitored in five different oocytes. Data are presented as mean ± S.E.M.

induce inward currents in the same oocyte (Fig. 3). In fact, ibuprofen and ketoprofen induced small, but significant, outward currents (Table III). These data indicate the absence of any appreciable Na^+/NSAID cotransport in hSMCT1-expressing oocytes as detectable by the electrophysiologic approach.

Studies with the mammalian cell expression system showed that ibuprofen and other related NSAIDs inhibited nicotinate uptake via hSMCT1. Because these drugs are not transportable substrates, if they inhibit nicotinate uptake by competing for the substrate-binding site and consequently blocking the binding of nicotinate to the transporter, these drugs should be able to interfere with substrate-induced inward currents in SMCT1-expressing oocytes. This was indeed the case. In the absence of ibuprofen, propionate (2.5 mM) induced marked inward currents in hSMCT1-expressing oocytes (Fig. 4A). However, when ibuprofen (2.5 mM) was included along with propionate in the perfusion medium, the currents induced by propionate were completely abolished (Fig. 4B). With a similar approach, we investigated the dose–response relationship for the blockade of propionate-induced currents by ibuprofen (data not shown). The magnitude of blockade increased with increasing concentrations of the inhibitor, with a $K_{0.5}$ value (i.e., concentration of ibuprofen necessary for half-maximal inhibition) of 11 ± 4 μM at −50 mV. We then analyzed the influence of Na^+ on the ability of ibuprofen to block the propionate-induced currents. This was performed by monitoring the ibuprofen (1 mM)-dependent blockade of propionate (2.5 mM)-induced currents in the presence of varying concentrations of Na^+. Because the magnitude of propionate-induced currents was dependent on the concentrations of Na^+, currents were

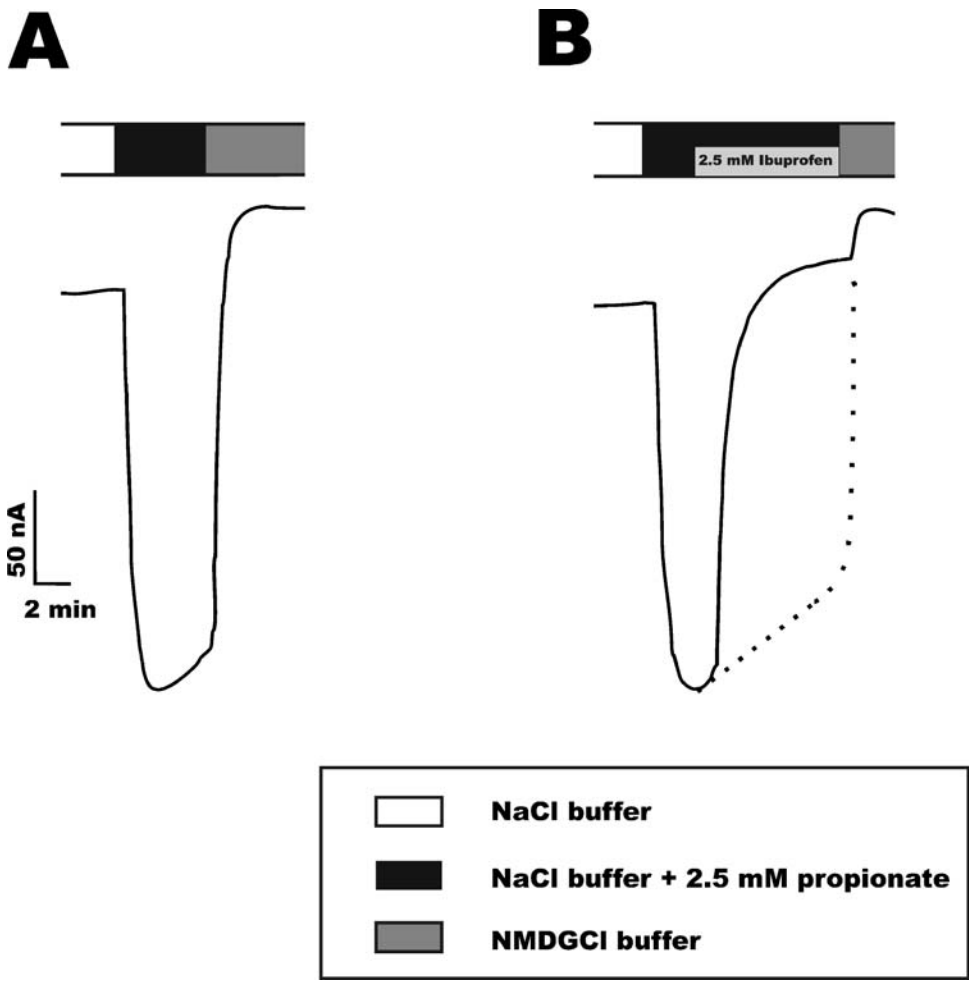

Fig. 4. Blockade of propionate-induced currents by ibuprofen. (A) hSMCT1-expressing oocytes were perfused with 2.5 mM propionate in the presence of NaCl. (B) hSMCT1-expressing oocytes were perfused, in the presence of NaCl, with 2.5 mM propionate and then with 2.5 mM propionate plus 2.5 mM ibuprofen.

measured at each concentration of Na^+ with or without ibuprofen, so that ibuprofen-dependent blockade could be determined at different concentrations of Na^+. These studies showed that the ability of ibuprofen to block the propionate-induced currents was dependent on the presence of Na^+ (Fig. 5). Analysis of the Na^+-dependence kinetics of this effect yielded a value of 3.1 ± 0.6 mM for $K_{0.5}^{Na}$ (i.e., concentration of Na^+ necessary to elicit half-maximal blockade of propionate-induced currents by ibuprofen). The Hill coefficient (h) for this process was 1.1 ± 0.1, suggesting that one Na^+ is involved in the interaction of ibuprofen with the transporter. We then performed the kinetics of the inhibitory effect of ibuprofen (25 μM) on hSMCT1-mediated propionate transport using the propionate-induced currents as the measure of transport activity (data not shown). Ibuprofen inhibited hSMCT1-mediated propionate transport in a competitive manner (K_i = 28 ± 4 μM), decreasing the affinity of the transporter for propionate without affecting the maximal currents induced by propionate. We also investigated the influence of Cl^- and H^+ on the ibuprofen effect (data not shown). The inhibitory effect of ibuprofen on the propionate-induced current was not affected when the experiments were performed in the absence of Cl^-, showing that Cl^- has no influence on the ibuprofen effect. While investigating the effect of pH, we found that propionate-induced currents were decreased significantly (~35%) at pH 6 as compared with pH 7.5. Similarly, ibuprofen effect was also decreased by ~25% at pH 6 as compared with pH 7.5. These pH effects are at least partly caused by the protonation of propionate and ibuprofen at pH 6 (pKa for propionate, 4.9; pKa for ibuprofen, 4.5). It is also likely that acidic pH affects the activity of the transporter directly to a significant extent.

The Hill coefficient of 1.1 ± 0.1 for the Na^+ activation of ibuprofen interaction with the transporter was surprising because the corresponding value was at least 2 for transportable substrates (2,4,5). If only one Na^+ is involved in the interaction of ibuprofen with the transporter, even if the drug is transported into oocytes via the transporter in a Na^+-coupled manner, the transport process would be electroneutral. Therefore, the electrophysiologic method used here would not be able to detect the transport of the drug. To determine unequivocally whether or not ibuprofen is transported into oocytes via the heterologously expressed hSMCT1, we used radiolabeled ibuprofen. These studies showed that the uptake of ibuprofen was not different between water-injected oocytes and hSMCT1-expressing oocytes, either in the absence or presence of Na^+ (Fig. 6A). Under identical conditions, the Na^+-dependent uptake of nicotinate was demonstrable in hSMCT1-expressing oocytes (Fig. 6B).

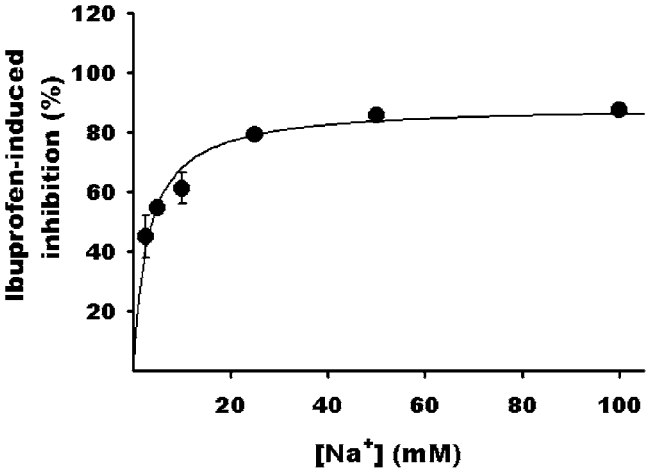

Fig. 5. Na^+ dependence of ibuprofen-induced blockade of propionate-induced currents. Blockade of propionate (2.5 mM)-induced currents by ibuprofen was measured in hSMCT1-expressing oocytes in the presence of increasing concentrations of Na^+ (2.5–100 mM).

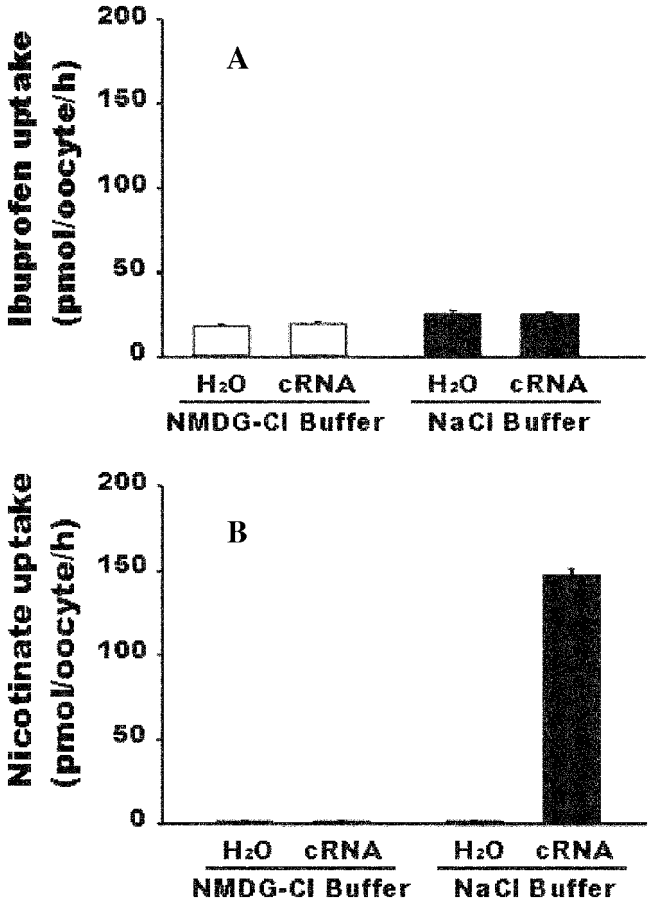

Fig. 6. Uptake of ibuprofen and nicotinate in hSMCT1-expressing oocytes. Uptake of [^{3}H]-ibuprofen (15 μM) (A) and [^{14}C]-nicotinate (15 μM) (B) was measured in water-injected oocytes and in hSMCT1-expressing oocytes.

DISCUSSION

The results of the present study show that all four NSAIDs tested (ibuprofen, fenoprofen, ketoprofen, and naproxen) interact with hSMCT1. This is evident from the ability of these drugs to interfere with the transporter-mediated uptake of nicotinate in a mammalian cell expression system. The IC_{50} values for the inhibition vary in the range of 25–120 μM. Fenoprofen is the most potent inhibitor,

with an IC_{50} value of 27 ± 6 μM. The corresponding value for ibuprofen was 64 ± 16 μM. The interaction of the drugs with SMCT1 is dependent on the presence of Na^+. These data suggest that ibuprofen and other structurally related NSAIDs interfere with SMCT1-mediated nicotinate uptake by interacting with the substrate-binding site of the transporter in a Na^+-dependent manner.

We then used the *X. laevis* oocyte expression system to investigate whether these NSAIDs are transportable substrates for SMCT1. These studies have yielded interesting results. None of the four NSAIDs examined is transported via SMCT1. This is evident from the lack of inward currents in SMCT1-expressing oocytes when exposed to these drugs. Although these drugs do not induce inward currents, when present together with propionate, they markedly reduce the currents induced by propionate. In other words, these drugs block the transport of propionate via SMCT1. This blockade is dose dependent with respect to the drug, with a K_i value in the range of 10–30 μM depending on the experimental method used. Kinetic analysis shows that the inhibition of propionate-induced currents by ibuprofen is competitive. The presence of Na^+ is essential for the ability of ibuprofen to block the transport of propionate. Interestingly, the dependence of ibuprofen's inhibitory effect on Na^+ indicated that only one Na^+ is likely to be involved in the interaction of the drug with the transporter. These data are different from those obtained with transportable substrates (2,4,5) because a Na^+/ibuprofen stoichiometry of 1:1 would mean that, even if ibuprofen is actually transported via SMCT1, the process would be electroneutral. Therefore, the absence of ibuprofen-inducible currents does not show definitively that this drug is not a transportable substrate. This necessitated the use of radiolabeled ibuprofen to monitor the uptake of the drug directly. These studies showed unequivocally that ibuprofen is not a transportable substrate for SMCT1. Taken collectively, the data show that ibuprofen is a blocker of SMCT1 and that the drug interferes with the transport function of the transporter by interacting with the substrate-binding site in a Na^+-dependent manner. We hypothesize that the other structurally related drugs (fenoprofen, ketoprofen, and naproxen) also behave in a similar manner in their interaction with SMCT1. The ability of ibuprofen to block the transport of propionate via the transporter is not influenced by Cl^-, but it is reduced to a significant extent (~25%) at pH 6 as compared with pH 7.5.

Coady *et al.* (3) have reported that the activity of the heterologously expressed SMCT1 in *Xenopus* oocytes exhibited temporal changes, with the activity decreasing significantly as the oocytes were exposed to SMCT1 substrates repeatedly. We did not observe such effects under our experimental conditions. The magnitude of propionate (2.5 mM)-induced currents was maintained at least for 1 h. Following the exposure of the oocytes to SMCT1 substrates for activity measurements, we perfuse the oocytes in a Na^+-free buffer in the absence of SMCT1 substrates until the baseline currents are returned and then continue the perfusion with a Na^+-containing buffer for at least 2.5 min prior to resuming the measurement of substrate-induced currents.

Previous studies have shown that ibuprofen and other structurally related NSAIDs are recognized as transportable substrates by MCTs (17,18). MCTs and SMCT1 exhibit no significant homology in terms of amino acid sequence; yet, these transporters show similar substrate selectivity. Therefore, it is interesting that the interaction of ibuprofen and other NSAIDs differs between MCTs and SMCT1 in that these drugs are transportable substrates for MCTs but are blockers of SMCT1.

These findings have biologic and pharmacologic significance. SMCT1 is expressed abundantly in the colon, kidney, and thyroid gland. In the colon, the transporter functions in the absorption of short-chain fatty acids as well as nicotinate. In the kidney, it may be involved in the reabsorption of lactate and nicotinate. In the thyroid gland, the function of the transporter may be in the release of iodide from the thyrocytes into the colloidal lumen. The blockade of the transport function of SMCT1 by ibuprofen and other structurally related NSAIDs may have biologic consequences in these tissues. Short-chain fatty acids, produced in the colonic lumen by bacterial fermentation, are known to offer protection against colorectal cancer (11). The beneficial effect of these bacterial metabolites depends on their entry into colonocytes, which is at least partly mediated by SMCT1. Chronic use of NSAIDs may interfere with the entry of short-chain fatty acids into colonocytes and thus compromise the beneficial effects. NSAIDs are known to have a protective effect against colorectal cancer because of their ability to inhibit cyclooxygenases (19,20). The beneficial effects of ibuprofen and other structurally related NSAIDs are evident in spite of the ability of these drugs to interfere with the entry of short-chain fatty acids into colonocytes. Therefore, if NSAIDs could be designed such that they do not block SMCT1 function but retain their inhibitory effect on cyclooxygenases, such drugs may have a more pronounced protective effect against colorectal cancer.

Niacin (nicotinate) is used widely as a lipid-lowering drug (21,22). The ability of SMCT1 to transport nicotinate in a Na^+-coupled manner indicates that the transporter plays a role in the active absorption of this drug in the intestinal tract. Nicotinate is not only a drug but also a vitamin. Dietary as well as bacteria-derived nicotinate may be absorbed in intestine and colon actively via SMCT1. Chronic use ibuprofen and other related NSAIDs may compromise the intestinal and colonic absorption of this drug/vitamin. In individuals who take niacin as a drug, concomitant use of NSAIDs may reduce the oral bioavailability of the drug and consequently compromise its therapeutic effects. In individuals who do not take niacin as a drug, chronic use of NSAIDs may cause niacin deficiency by interfering with the intestinal and colonic absorption of this vitamin. It has been shown that high dosage of ibuprofen taken consistently for 4 years results in peak serum concentrations of 50–100 mg/L (250–500 μM) (23). Similarly, the plasma levels of fenoprofen achieved by therapeutic doses are in the range of 60–200 μM (24). Because ibuprofen and fenoprofen block the transport function of SMCT1 at micromolar concentrations, we speculate that these drugs would effectively compromise the physiologic functions of the transporter in tissues such as the kidney and thyroid gland. In such instances, the concentrations of ibuprofen and fenoprofen in the intestine and colon are also likely to be quite high, making the transporter in the intestinal tract vulnerable for inhibition.

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